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EARLY INFANT DIAGNOSIS OF HIV USING DNA PCR CYCLE THRESHOLD AND REPEAT TESTING ALGORITHM

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Background

- 90% of new pediatric HIV infections are acquired by vertical transmission from mothers to infants, most often *in utero* or in the immediate postpartum period
- Disease progression occurs rapidly in infants, but survival benefit has been demonstrated from starting ART early in life (CHER Study)
  -- *Earliest possible ART start recommended by WHO*
- Early infant diagnosis (EID) of HIV (immediately after birth) has several possible advantages:
  -- may improve testing uptake and help with retention
  -- may allow for rapid initiation of ART
  -- may limit disease progression and restrict viral reservoir seeding
- Limitations of EID include added cost (follow-up HIV testing later in life still required), and lack of a standardized testing algorithm for birth testing
- Confirmatory re-testing *after* ART begins is problematic, because loss of virologic positivity may occur on ART, and serologic positivity may not develop

-- **Diagnostic testing performed at birth needs to be highly specific**

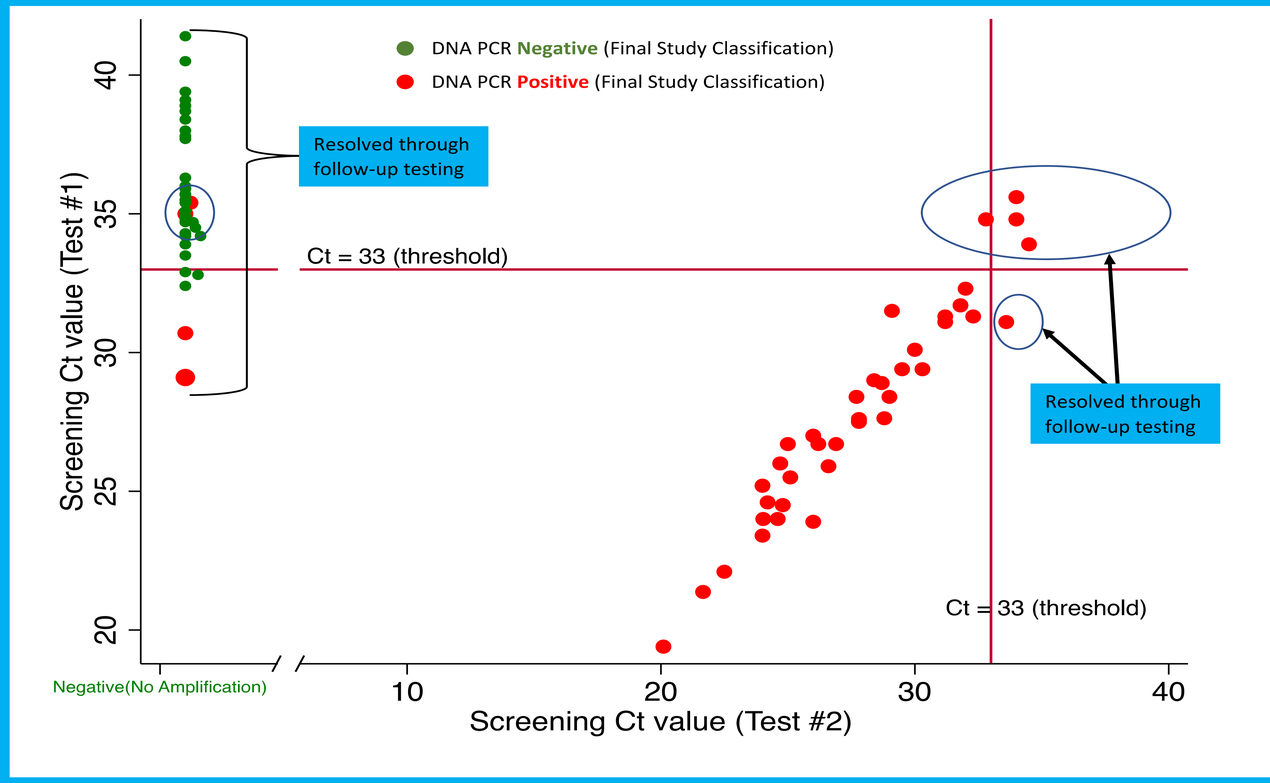
Methods

- From April 2015-July 2018, the Early Infant Treatment Study (EIT) screened HIV-exposed infants in Botswana at < 96 hours from delivery
- Qualitative DNA PCR test using the Roche TaqMan was conducted on all samples collected with a result turnaround time of 24 hours
- A negative DNA PCR test was defined as no HIV DNA amplification (target not detected) at initial dried blood spot screening
- A positive test was two spots from same sample with target detected at any cycle threshold (Ct) value
- An indeterminate test was discordant spots (target detected/target not detected) from same sample
- Repeat blood draw occurred for initial positive and indeterminate results
- Quantitative HIV-1 RNA testing occurred for those presumptively enrolled in the study
- We compared Ct values by the ultimate HIV status of the child (as confirmed by subsequent HIV-1 DNA, and when possible DNA/RNA, testing)

Results

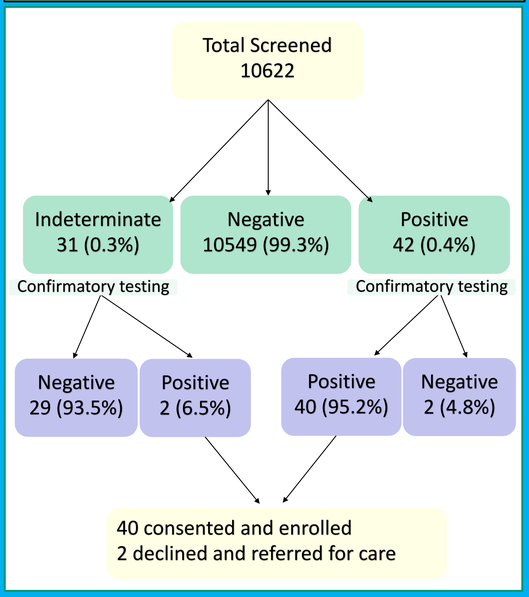
- 10622 HIV-exposed infants screened (Figure 1)
  - 10548 (99.3%) tested negative, 42 (0.4%) tested positive, and 31 (0.3%) tested indeterminate at the first HIV screening test
  - On repeat testing, 40 (95.2%) of the initial 42 positive infants remained positive and 2 (4.8%) tested negative
  - Of 31 indeterminates, repeat testing confirmed 29 (93.5%) as negative and 2 (6.5%) as positive
- **Confirmatory testing of all positives and indeterminates re-classified 4 (5.5%) infants in total**
  - 1 (1.4%) indeterminate required further HIV RNA testing to become reclassified as positive
- Median DNA PCR Ct value for positive results was 28.1 (range 19.4, 35.6) for the first screening spot and 28.3 (range 19.8, 36.2) for the second screening spot
- Median DNA PCR Ct value for indeterminate results was 35.5 (range 32.8, 41.4) for the first screening spot and 35.6 (range 34.4, 40.6) for the second screening spot
- **6 (8.2%) infants with final HIV+ status had Ct value > 33 at first screen (Figure 2)**
- **2 (6.5%) with final HIV-negative status initially had indeterminate result with a Ct value < 33**

Figure 2: Ct values for initial HIV DNA PCR screens with a target detected at first sampling. Spot #1 (target detected) shown on y-axis, Spot #2 (target either detected or not) shown on x-axis. Colors indicate final HIV status from follow-up confirmatory testing (confirmatory values not shown)



Note: Initial screening consisted of dried blood spot testing on a single spot (Test#1), with a second spot (Test #2) performed for all instances of HIV DNA target detection at any cycle threshold. Follow-up testing (results not shown) was performed on a second blood sample (using dried blood spot or plasma).

Figure 1: Screening flow diagram



Conclusions

- **A standard cycle threshold of 33 distinguished most true positives from true negatives in the first week of life, but confirmatory HIV DNA and RNA testing was needed to eliminate misclassification**
  - **95% identified as positive on initial screen were true positives**
  - **94% identified as indeterminate on initial screen were true negatives**
  - **False negatives on initial screen could not be determined from this study**
- **All positive and indeterminate HIV DNA PCR testing at birth should be confirmed with repeat testing**

Acknowledgements

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